THE EXPRESSION OF INOS IN MOUSE EXPERIMENTAL MODEL POINTS TO INFLAMMATORY CONDITIONS ASSOCIATED WITH PARKINSON'S DISEASE

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Abstract

Parkinson's Disease (PD) is one of the most common neurodegenerative diseases. Several molecular mechanisms are involved. The objective of conducting this study was to evaluate the expression of iNOS in mouse experimental model of PD. PD was induced through injecting mice with 10 doses of MPTP (25 mg/kg) and probenecid (250 mg/kg). Mice in control group were injected by saline (25 mg/kg). Immunohistochemical stains for iNOS in brain sections were carried out using indirect immunoperoxidase techniques. Study findings showed that there was a significant difference in the expression level iNOS in study groups ($P<0.001$), and experimental PD group had more expressed iNOS levels compared with control group. Taken together, the present study confirmed the impact of induction of iNOS in the etiology of PD.

Keywords:

Introduction

Parkinson's disease (PD) is a chronic-progressive and disabling neurological disorder (Tolosa, 2006). From a pathologic point of view, PD can be defined by nigrostriatal loss of dopaminergic cells and Lewy bodies in the surviving cells on autopsy. Furthermore, PD may be manifested from a clinical point of view by a broad spectrum of motor and non-motor features. The four cardinal features of PD can be grouped under the acronym TRAP: tremor at rest, rigidity, akinesia (or bradykinesia) and postural instability. This syndrome is labeled "parkinsonism" and may also occur in other medical conditions than idiopathic PD, such as dementia with Lewy bodies, cerebrovascular disease, the so called parkinsonian plus syndromes or as side effect after administration of neuroleptic medication. The presence of

akinesia and one of the other symptoms are considered sufficient for the clinical diagnosis of parkinsonism. Diagnostic criteria have been developed by the UK Parkinson's Disease Society Brain Bank and the National Institute of Neurological Disorders and Stroke (NINDS) (Tolosa, 2006). Other diagnostic criteria for clinical subgroups of the disease were suggested by Larsen et al (1994). According to a study conducted by Jankovic (2008), flexed posture and motor blocks (freezing) have been included among classic features of PD. It has been shown that the diagnosis of PD is still based on the presence of a combination of cardinal motor features, associated and exclusionary symptoms, and response to levodopa (Rao, 2003).

Nitric oxide (NO) is gas in nature and it is produced by nitric oxide synthase (NOS) family of enzymes from L-arginine (Seet, 2010; Juurlink, 1999). It is known as a highly reactive signaling molecule having a few seconds of life time (Juurlink, 1999). Furthermore, it diffuses with ease (Juurlink, 1999). Nitric oxide is membrane permeable and can diffuse into dopaminergic neurons (Ara, 1998). Thus NO receptors are signaling transduction for intracellular communication. Once generated, the cell cannot control the local concentration of NO. Calabrese et al., shows that the activity of NO can be influenced by the degree of its synthesis and the activity is terminated with its reaction with its substrate (Seet, 2010). This is implicated in many physiological and pathological processes within the mammalian body (Moncada, 1997).

It has been shown that a histopathological evidence that NO and glutamate in toxic dose may mediate certain neurodegenerative diseases (Przedborski, 1996). Postmortem studies, clinical findings, and evidence from experimental models revealed the role of NO in the degeneration of dopaminergic neurons in PD. Studies performed in the MPTP model of PD suggest that peroxynitrite, a reactive species formed by the nearly diffusion-limited reaction of nitric oxide with superoxide, may be a mediator of nigrostriatal damage in PD (Ara, 1998). Over limit NO could contribute to the formation of free radicals that could be involved in the death of dopaminergic neurons, resulting in development of PD symptoms (Tuncel, 2009). Excess NO synthesis is likely to be involved in the progressive neuronal loss that distinguishes PD (Hancock, 2008). However, there is significant reduction in NO level in PD patients than in controls. The explanations for these low levels may be a faulty NO-dependent adaptation mechanism or the depletion of NO storage during the course of PD (Tuncel, 2009).

Moreover, as cytokines enhance the induction of NOS in brain; many studies suggest the role of glial derived NO in the pathogenesis of these diseases. Some studies showed that there was excessive formation of NO of glial origin in which NADPH diaphorase (a cytochemical marker of NOS

activity) positive glial cells have been identified in the substantia nigra of postmortem brains obtained from individuals with Parkinson's disease (Seet, 2010).

Several animals and human studies showed that of the three isoforms, only the nNOS and iNOS are relevant with regard to their potential impact in neurodegeneration and glial response in PD (Kroncke, 1998). Several experiments showed that animals can be protected against MPTP by nNOS inhibitors (Seet, 2010; Kroncke, 1998). This gives evidence that the nNOS is responsible for MPTP neurotoxicity (Al-Jarrah, 2010). Furthermore, mutation in nNOS gene makes the mice more resistance to MPTP than wild-type mice. Similar experiments with iNOS show that this enzyme also plays a role in the dopaminergic neurons sensitivity (Kroncke, 1998). In human, overproduction of NO was detected in the substantia nigra of PD brain. It was correlated with high concentration of nNOS and iNOS (Al-Jarraha, 2010). Moreover, NOS inhibition protects against MPTP-induced loss of nigral neurons. Inhibition of NOS activity can stop MPTP-induced damage of dopaminergic nerve terminals in the striatum and the loss of dopaminergic cell bodies in the substantia nigra pars compacta (Dishman, 2006).

Study objectives

The main objective of the current study is to study the expression of iNOS in mice experimental model of PD using immunohistochemical techniques.

Methodology

Twenty Albino mice were selected randomly and assigned into 2 groups: Control group (N=10), PD group (N=10).

The animals were housed in individual cages under identical conditions (22 ± 1 °C, free access to standard chow and water, 12 hours dark/light cycle). PD was induced by injecting mice with 10 doses of MPTP (25 mg/kg) and probenecid (250 mg/kg) (chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA)). Mice in control group were injected by saline (25 mg/kg).

iNOS immunostaining of brain tissue

The mice were sacrificed, and their brains were removed and fixed in 10% formalin, embedded in paraffin, and sliced into 3 micometer thick sections. Then, the 3 μm thick sections were processed via immunohistochemistry using an antibody to iNOS (Santa Cruz biotechnology). So, the 3 micron thick paraffin-embedded sections mounted on glass slides were deparaffinized in xylene for 2 minutes twice, and subsequently rehydrated through serially descending dilutions of alcohol

(starting with 100%, and ended with 70%) followed by water (2 minutes for each step). After that, sections were processed for antigen retrieval in the reveal solution (RV 1000M, Biocare Medical, Concord, CA) under pressure in the Decloaking chamber (Biocare medical) for 2 minutes. Tissue sections were then cooled down to room temperature, and incubated with 3% hydrogen peroxidase in methanol for 5 minutes. After washing the sections in phosphate buffered saline (PBS), they were incubated with iNOS antibody (Santa Cruz Biotechnology), with the dilution recommended by the vendor, at room temperature for one hour. Next, the sections were washed in PBS and treated with secondary antibodies and Streptavidin using ImmunoCruz™ goat ABC Staining System (sc-2023). Diaminobenzidine (DAB) was applied for 2 minutes or longer, until the desired intensity was developed, and then the slides were washed with tap water to stop the reaction. Negative control sections were processed without the primary antibody. All sections were then counterstained with hematoxylin and viewed under the light microscope. Ten slides of brain tissues from each animal group were evaluated for iNOS expression by immunohistochemistry.

Data analysis

The sections were photographed with digital camera. Photoshop software was used. The slides from each group were analyzed by counting the total pixels area occupied by positive staining. iNOS expression was analyzed, in the different brain tissues, and statistically compared among the 2 different groups using paired student t-test. Differences in iNOS expression were considered statistically significant at P value < 0.05.

Results

The expression of iNOS in study groups

The study findings showed that the expression level of iNOS in control group was 0.125 and this expression level was further increased in Parkinson's disease group, 0.21 (Figure 1). This variation in the expression level in study groups was statistically significant ($P< 0.001$) .

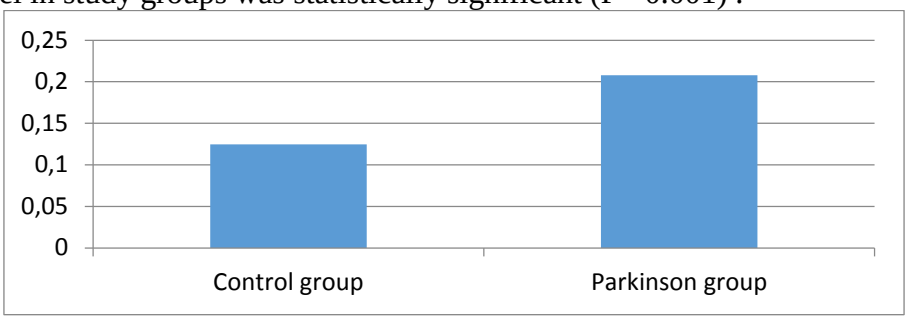

Figure 1: comparison of expression level of iNOS among study groups

Discussion

The present study was conducted to evaluate the expression level of iNOS in PD compared with control group.

The results of the present study clearly identified a significant involvement of iNOS in the etiology of PD. This finding agrees with other studies in which it was found that iNOS has impacts in neurodegeneration and glial response in PD and plays a role in the dopaminergic neurons sensitivity (Kroncke, 1998).

The increase of iNOS is possibly due to the release of cytokines and other immunity mediators, which induce iNOS. Moreover, Husain and Hazelrigg (2002) found that the exercise training and chronic NOS inhibitor (nitro-L-arginine methyl ester, LNAME) administration lead to a significant induction of iNOS after exercise in heart of rats. This may be due to increase blood flow during exercise which leads to the upregulation of iNOS, or may be due to positive effect of exercise on catecholamines, and hence upregulation of iNOS expression.

In human, overproduction of NO was detected in the substantia nigra of PD brain. It was correlated with high concentration of nNOS and iNOS (Al-Jarraha, 2010). Moreover, NOS inhibition protects against MPTP-induced loss of nigral neurons. Inhibition of NOS activity can stop MPTP-induced damage of dopaminergic nerve terminals in the striatum and the loss of dopaminergic cell bodies in the substantia nigra pars compacta (Dishman, 2006).

Conclusion

The present study confirmed the impact of induction of iNOS in the etiology of PD.

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